III. AMENDMENT

PLEASE ENTER THE FOLLOWING AMENDMENT WITHOUT PREJUDICE OR DISCLAIMER. Applicants reserve the right to file a divisional or continuation application to the originally filed claims.

(i) Amendment To The Claims:

Please amend the claims as follows:

1. (Original) A method for the analysis of organisms, cells or both organisms and cells; said method comprising:
    a) collecting a sample of organisms or cells;
    b) adding one or more fixative agents to the sample to thereby fix the organisms, cells or both;
    c) treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with the molecular probe in a way that produces detectable or independently detectable organisms, cells or both; and
    d) determining one or more of the detectable organisms or cells in the sample; wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination.

2. (Original) The method of claim 1, wherein the organisms, cells or both are collected from a growth medium.

3. (Currently Amended) The method of claim 1, wherein the organisms, cells or both are collected directly from a sample that has not been treated ~~for growth~~ with a growth medium.

4. (Currently Amended) The method of claim 2, wherein the growth medium is not completely separated from the sample of organisms, cells or both.

5. (Original) The method of claim 2, wherein the growth medium is selected from the group consisting of broth and agar.

6. (Original) The method of claim 1, wherein a blocking agent is present during the operation of step (c).

7. (Original) The method of claim 6, wherein the blocking agent is casein.

8. (Original) The method of claim 1, wherein steps (b) and (c) are performed simultaneously.

9. (Original) The method of claim 1, wherein steps (b) and (c) are performed sequentially in that order.

10. (Original) The method of claim 1, wherein the molecular probe is labeled with a fluorophore.

11. (Original) The method of claim 1, wherein two or more independently detectable molecular probes are used in the method for the multiplex analysis of two or more different types of organisms or cells in the sample.

12. (Original) The method of claim 11, wherein the two or more independently detectable molecular probes are labeled with independently detectable fluorophores.

13. (Original) The method of claim 1, wherein the molecular probe is a self-indicating molecular probe selected from the group consisting of a linear beacon, a nucleic acid or PNA molecular beacon and an intercalating beacon.

14. (Original) The method of claim 1, wherein the molecular probe is a detection complex.

15. (Original) The method of claim 1, further comprising:

    e) adding a quencher labeled oligomer before the determination is made to thereby form a complex between the excess molecular probe and the quencher labeled oligomer.

16. (Original) The method of claim 1, wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer.

17. (Original) The method of claim 1, wherein one or more blocking probes are present during the operation of step (c).

18. (Original) The method of claim 1, wherein the molecular probe is a nucleic acid probe.

19. (Original) The method of claim 1, wherein the molecular probe is a non-nucleic acid probe.

20. (Original) The method of claim 19, wherein the non-nucleic acid probe is a peptide nucleic acid probe.

21. (Currently Amended) A method for determining organisms, cells or both, said method comprising:

    a) treating a sample of fixed cells, organisms or both, that have been grown in a medium, with one or more detectable molecular probes, under suitable hybridization conditions, in a way that produces stained organisms, cells or both stained organisms and cells; and

    b) determining the stained cells, organism or both the stained organisms and cells;

wherein ~~said assay does not require that~~ the medium ~~be~~ is not removed or separated from the organisms, cells or both the organisms and cells.

(ii) Remarks On The Amendment To The Claims:

Amendments to claims 3 and 21 are made to address the rejections under 35 U.S.C. §112, second paragraph that were articulated by the Examiner at pages 20-21 of the present Office Action.

It is respectfully submitted that the amendment does not introduce new matter.

IV. RESPONSE TO THE OTHER OFFICE ACTION REJECTIONS

1. Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 3 and 21 have been amended in response to the rejections made under 35 U.S.C. § 112, second paragraph. Accordingly, it is believed that the amended claims render moot these rejections.

2. Rejection Under 35 U.S.C. § 102(b)

(a) Statement Of The Law Of 35 U.S.C. § 102(b)

It is well settled that to be anticipated, a prior art reference must teach each and every element/limitation of the claimed subject matter. M.P.E.P. § 2131. Moreover, the elements must be arranged as required by the claim. *Id.* "The identical invention must be shown in as complete detail as is contained in the claim" *Id.* quoting from *Richardson v. Suzuki Motor Co.*, 868 F2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

(b) The Teachings Of Yurov et al. (Hum. Genet 97: 390-398 (1996)

Claim 1 recites: "... wherein the fixative agent or agents **and** excess molecular probe or probes are not separated from the organisms or cells prior to making the determination (emphasis added)." In view of the use of the contraction "and" both of the conditions must be met. Thus, if either one or both of the fixative agent or agents and the excess molecular probe or probes are separated from the organisms or cells prior to making the determination, the elements/limitations of the claim have not been fulfilled.

In the present Office Action, the Examiner appears to take the position that Yurov et al. (Hum. Genet 97: 390-398 (1996)) teaches an *in-situ* hybridization assay wherein both the fixative agent or agents **and** excess molecular probe or probes are not separated from the organism or cells prior to making the determination of: "...one or more of the detectable organisms or cells in the sample ...". Applicant's respectfully must disagree with the Examiner's opinion of what the reference teaches.

At top of page 3 of the Office Action, the Examiner makes reference to "(m&m (materials and methods), Pg 391 on bottom right and Pg. On top left)" as support for the position that the reference teaches: "d) determining one or more of the detectable organisms or cells in the sample (391); wherein the fixative agent or agents and excess

molecular probe or probes are not separated from the organisms or cells prior to making the determination..." .

At page 391 the method used for performing the *in-situ* hybridization assay is described under the heading: "In situ hybridization and probe detection". In the first line of this description Yurov et al. state: "In situ hybridization was performed as described in detail previously for isotopic in situ hybridization **(Yurov 1984; Yurov et al. 1987)"** (emphasis added). These two documents have been included in the IDS filed with this response. Accordingly, it is essential to review the materials and methods sections of these two references in order to fully appreciate the process that Yurov et al. (Hum. Genet 97: 390-398 (1996)) applied in the experiments they performed.

Review of the experimental section of the cited Yurov 1984 document reveals that several wash steps were performed between the step of fixation and the step of determining the chromosomes in the cells. Accordingly, it is clear that these wash steps separated the fixative agent or agents and/or excess radiolabeled probe from the cells or organisms prior to performing the determination step. Similarly, a review of the experimental section of the cited Yurov et al. 1987 document reveals that there was a treatment with 0.07 N NaOH for 30 s between the step of fixation and the step of determining the chromosomes in the cells. Accordingly it is again clear that this treatment would separate the fixative agent or agents and/or excess radiolabeled probe prior to performing the determination step.

In addition to the foregoing, the materials and methods section of Yurov et al. (Hum. Genet 97: 390-398 (1996)) specifically describes the following steps be performed between the fix and the DNA probe hybridization step(s).

> "Slides with fixed cells (blood lymphocytes and aminocytes) were treated with 0.07 N NaOH, 2 x SSC for 30 s for chromosomal DNA denaturation, dehydration in 70%, 96%, 100% ethanol solutions for 2 min each, and air-dried. Slides with interphase tumor cells, spermatozoa nuclei, or buccal epithelium cells were treated in 2 N NaOH, 2 x SSC for 2-3 min without application of pronase or pepsin treatment, dehydration in ethanol and air-dried."

From this description it is clear that the fixative agent or agents that have been used to fix the cells or organisms are indeed separated from the cells or organisms by the process of treatment with the 2 N NaOH, 2 x SSC followed by the dehydration process; all these steps being performed before hybridization with the molecular probe or probes or the step of determination.

In view of the foregoing, it is clear that Yurov et al. (Hum. Genet 97: 390-398 (1996)) does not anticipate the subject matter of independent claim 1, and its associated dependent claims because this document simply does not teach all of the elements/limitations of the claimed subject matter arranged as required by the claims.

3. Rejections Under 35 U.S.C. § 103(a)

All remaining rejections articulated in the present Office Action are made under 35 U.S.C. § 103(a) and are cumulative with respect to the rejection under 35 U.S.C. §102(b). In particular, they all necessarily require the Examiner's interpretation of the Yurov et al. (Hum. Genet 97: 390-398 (1996)) reference with which the Applicant's take issue. Because it is believed that the present rejection under 35 U.S.C. §102(b) is not proper, it is believed that all of the rejections under 35 U.S.C. §103(a) must properly be withdrawn.

V. SUMMARY

It is believed that this response addresses all rejections set forth in the present Office Action and the application is in ready condition for allowance. In consideration of the preceding amendments and remarks, Applicants hereby respectfully request reconsideration of all pending claims (as amended), the withdrawal of all rejections set forth in the present Office Action and issue of a Notice of Allowance by The Office.

VI. INTERVIEW

If the Examiner believes a telephonic or personal interview would advance the prosecution of the subject application, the Examiner is invited to contact attorney Gildea during business hours at the telephone or facsimile numbers listed below.

VII. FEES

The petition under 37 C.F.R. §1.136(a) that accompanies this paper includes an authorization to deduct the appropriate fee from Deposit Account 02-3240. No additional fees are believed due The Office for consideration of this paper. If however, The Office determines that any other fee is due, authorization is hereby granted to charge any required fee associated with the filing or proper consideration of this paper to Deposit Account 02-3240.

VIII. CORRESPONDENCE/CUSTOMER NUMBER

Please send all correspondence pertaining to this document to:

Applied Biosystems
Attn: Brian D. Gildea, Esq.
15 DeAngelo Drive
Bedford, MA 01730
Telephone: 781-280-2824
Fax: 781-280-2940

IF NOT ALREADY DONE, PLEASE MATCH THIS CASE WITH CUSTOMER NUMBER **23544**

[Insert Customer Number Bar Code]

Respectfully submitted
on behalf of Applicants,

July 23, 2003
Date

Brian D. Gildea, Esq.
Reg. No. 39,995